

CLAIMS :

1. A method for the determination and individual characterization of particles by means of at least two different detectable probes in a sample is proposed, wherein
 - the particles, especially molecules or molecular aggregates, have at least one binding site, preferably a multitude of binding sites, for at least one of said at least two different detectable probes;
 - said at least two different detectable probes are present in the sample;
 - a measure of the number of bound probes and
 - the mutual ratio of bound probes are established by determining particles;
 - said determining being effected on the basis of single particles.
2. The method according to claim 1, characterized in that the mutual ratio of bound probes is established by determining particles in a measuring volume which is a subvolume of the sample to be examined.
3. The method according to claim 2, characterized in that the determination is on the basis of single particles which are within the measuring volume at different times.
4. The method according to any of claims 1 to 3, characterized in that the detection of different bound probes is effected simultaneously on one particle.

5. The method according to any of claims 1 to 4, characterized in that the measuring volume is $\leq 10^{-12}$ l, especially $\leq 10^{-14}$ l.
6. The method according to any of claims 1 to 5, characterized in that the measurement is performed using a confocal microscopic set-up, a near-field set-up or a set-up for multiphoton excitation.
7. The method according to any of claims 1 to 6, characterized in that the determination and characterization of particles is effected in a homogeneous assay method without washing steps.
8. The method according to any of claims 1 to 7, characterized in that the quantification of the particle-caused signal fraction is preferably effected by analyzing the intensity distribution of a measured detection signal, especially a fluorescence signal, in successive time windows with detection times of constant or variable lengths in the range of micro- to milliseconds.
9. The method according to claims 1 to 8, characterized in that an intensity-based separation of the particle-caused signal fraction is effected by an algorithm for peak detection and analysis.
10. The method according to any of claims 1 to 9, characterized in that scanning of the sample is effected by producing an essentially constant relative movement between the sample and measuring volume.
11. The method according to claim 10, characterized in that the relative movement is realized by a lens system which allows for movement of the measuring volume, by movement of a sample carrier holding the sample, or by a flow capillary.
12. The method according to any of claims 1 to 11, characterized in that antibodies are used as probe molecules.

13. The method according to any of claims 1 to 12, characterized in that simultaneous analysis of two or more probes, especially fluorescent probes, which are separately measurable in the same measuring volume and emitting in different wavelength regions or polarization planes is effected.
14. The method according to claim 13, characterized in that data from multiple color measurements, especially dual color measurements, or multiple polarization measurements are established and optionally arranged in a multidimensional, especially two-dimensional, array for evaluation, for example, arranged as an intensity histogram.
15. The method according to any of claims 1 to 14, characterized in that pathological protein aggregates are detected as particles, especially prion proteins by subspecies, are detected by labeling them with probe molecules.
16. The method according to claim 15, characterized in that the binding of at least two different probe molecules to the particles forming the protein aggregates is detected, and the subspecies is determined from the mutual ratio of amounts of different bound probe molecules.
17. The method according to any of claims 1 to 16 for pathogenic strain typing or for examining the relative binding of proteins from different species to pathological protein aggregates of a particular species for estimating an interspecific barrier for the transmission of a disease.
18. The method according to any of claims 1 to 17 for the examination of degenerative diseases, especially neurodegenerative diseases, with formation of pathological aggregates, especially aggregates which contain prion protein, APP, Tau, synuclein or proteins having a polyglutamine sequence, such as huntingtin, or fragments or derivatives of such proteins as a component.

19. The method according to any of claims 1 to 18 for examining subcellular particles, especially for the phenotypical analysis of viral particles, or for analyzing nucleic acids using antisense probes.